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Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent exposition vector.

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Practically any oligopeptide can be exposed on the surface of the bacteriophage capsid by fusion to the major coat protein of filamentous bacteriophages. A phage expressing a particular peptide tag can be selected from a mixture of tens of millions of clones, exposing oligopeptides of random sequence, by affinity purification with a protein ligand. In this respect, pVIII can be used as an alternative and complement to the expositior vectors based on the product of gene III (pIII). We have constructed a phagemid vector that contains gene VIII under the control of the pLac promoter. This vector can be conveniently used to construct libraries of oligopeptides with a random amino acid sequence. An antipeptide monoclonal antibody was used to affinity-purify phagemids exposing oligopeptides which can interact with the monoclonal antibody. DNA sequencing of the amino terminus of gene VIII of the recovered clones predicts the synthesis of hybrid proteins whose aminoterminal amino acid sequence is related to that of the oligopeptide used to raise the antibody. In other words, only oligopeptides that bind a very small portion of the immunoglobulin G surface are affinity-purified by this method, implying tha the antigen binding site possesses molecular properties that renders it much stickier than the remainder of the molecule.

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cDNA clones encoding immunoglobulin lambda chains from rabbit expressing the phenotype c7.

Hayzer DJ, Young-Cooper GO, Mage RG, Jaton JC.

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A cDNA library derived from spleen cells of an unimmunized rabbit expressing the c7 phenotype of Ig lambda chains (c7+, c21-) was screened with V lambda or C lambda probes of a lambda light chain bearing c21 epitopes. The nucleotide sequences of three hybridizing clones were found tc be identical within the V lambda, J lambda and C lambda regions. The V lambda region was 97% similar to that of the functional germ-line gene V lambda 2, and the C lambda region was identical to that of gene C lambda 6, recently identified. Gene C lambda 6 exhibited four codon differences when compared with gene C lambda 5, the latter encoding c21 epitopes. The data presented here and in the accompanying report (Jaton, J.-C. et al., Eur. J. Immunol. 1990, 20:2713) support the view that gene C lambda 6 encodes the C region of c7 lambda chains and that c7 and c21 markers designate two distinct isotypic forms of lambda chains. On the basis of comparative Southern blotting analyses and restriction maps of cloned genomic regions containing V lambda and C lambda genes, a scheme is proposed to account for the c7- and c21- phenotypes.

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1: J Immunol 1989 Oct 15;143(8):2730-9 Related Articles, Link

Nucleotide sequence of channel catfish heavy chain cDNA and genomic blot analyses. Implications for the phylogeny of Ig heavy chains.

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Our prior analyses defined the cDNA sequence on part of the CH2 domain, the complete CH3 and CH4 domains, and the 3'-untranslated region of a catfish H chain. To complete the catfish H chain mRNA sequence, a primer-extended H chain cDNA library was constructed. Analysis of this library has resulted in the definition of full-length clones encoding a 61-bp 5' untranslated region, a 51-bp leader sequence, the V region and the complete CH1 and CH2 domains. The high similarity defined with other vertebrate V regions readily allowed the catfish sequence to be divided into FR and CDR regions. Sequence comparisons with mammalian VH and JH genes strongly suggest that the catfish V region is the product of multiple genes. Using a catfish VH cDNA probe, at least 25 different genomic VH members were defined. Because this probe does not hybridize with other full-length H chain cDNA clones, additional VH families will likely be defined in catfish. Phylogenetic sequence comparisons of the catfish C region domains indicated that the CH1 and CH4 were the most highly conserved. In addition several important features were defined in genomic Southern blot analyses of catfish DNA. Gene titration experiments established that the catfish CH gene is represented by a single genomic copy. This finding provides clear evidence that the genomic organization of H chain genes in catfish must be different from that defined in sharks and suggests that the phylogeny of single copy CH genes may have been established at the level of the bony fishes. It is also likely that there is an additional CH gene in catfish. This gene is also represented by a single genomic copy, and based upon its relative signal intensity when compared with the known CH gene it appears to share higher similarity with the known CH1 domain than it does with the CH2 domain.

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